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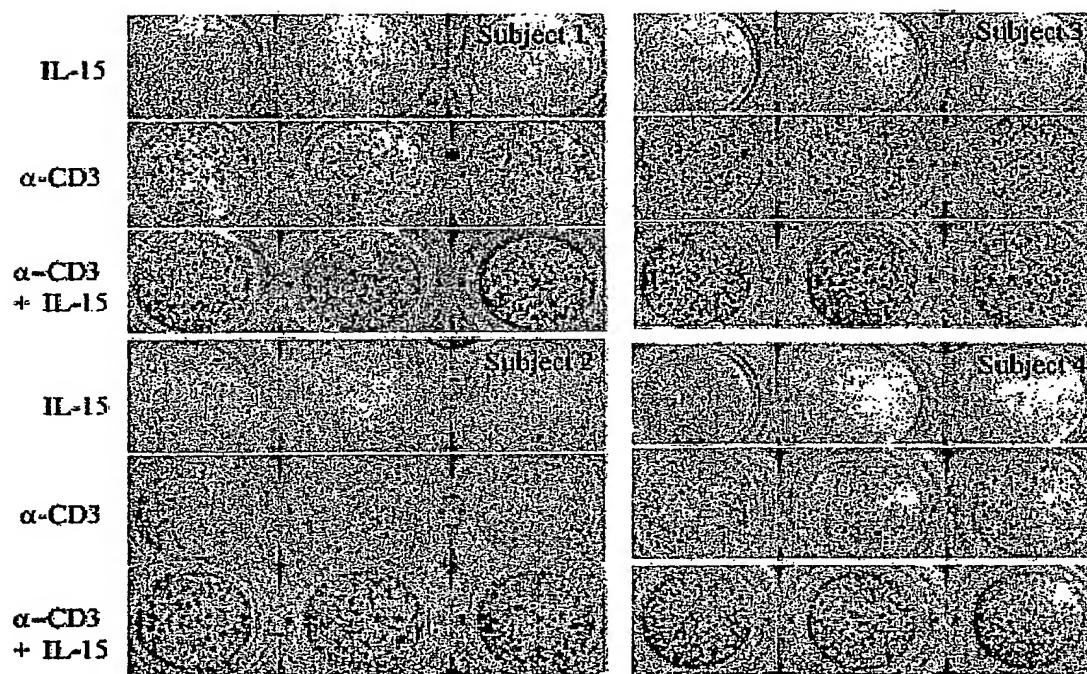


Figure 1

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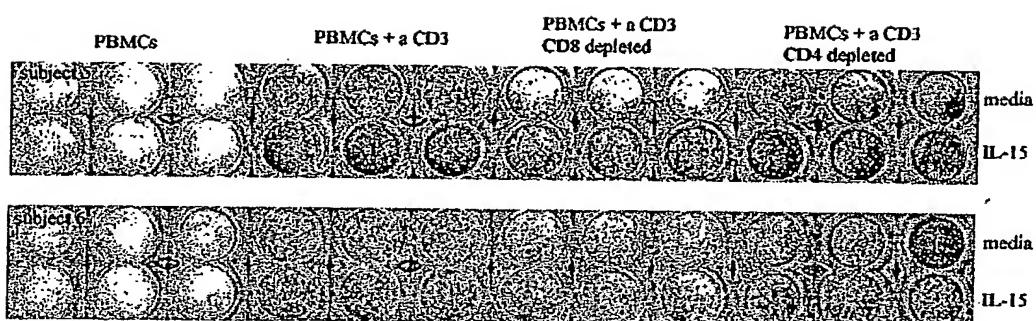


Figure 2

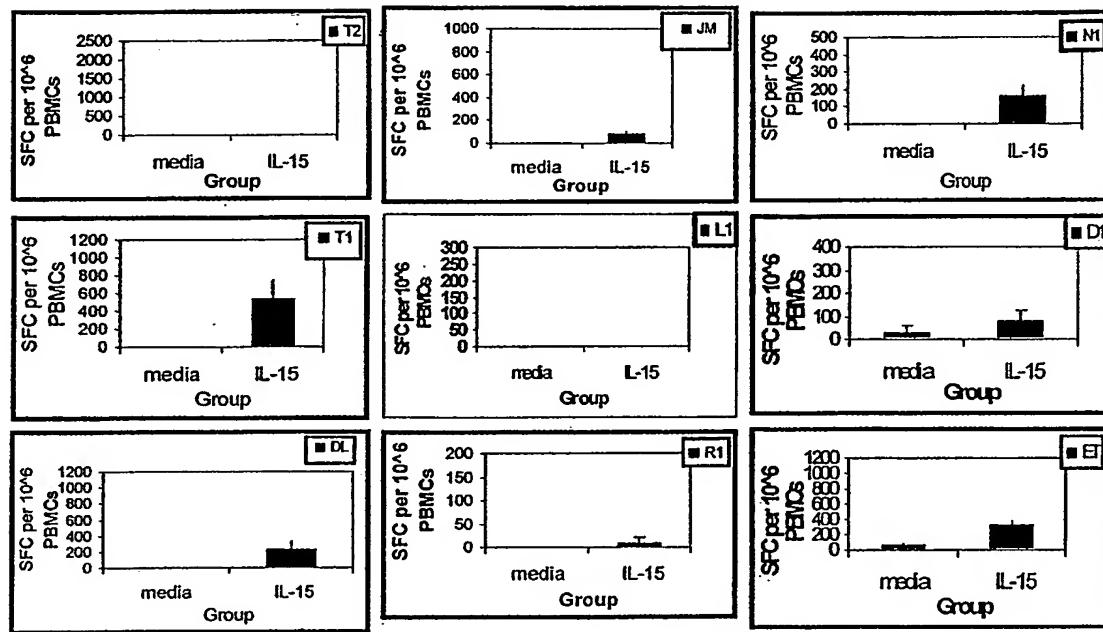


FIGURE 3A

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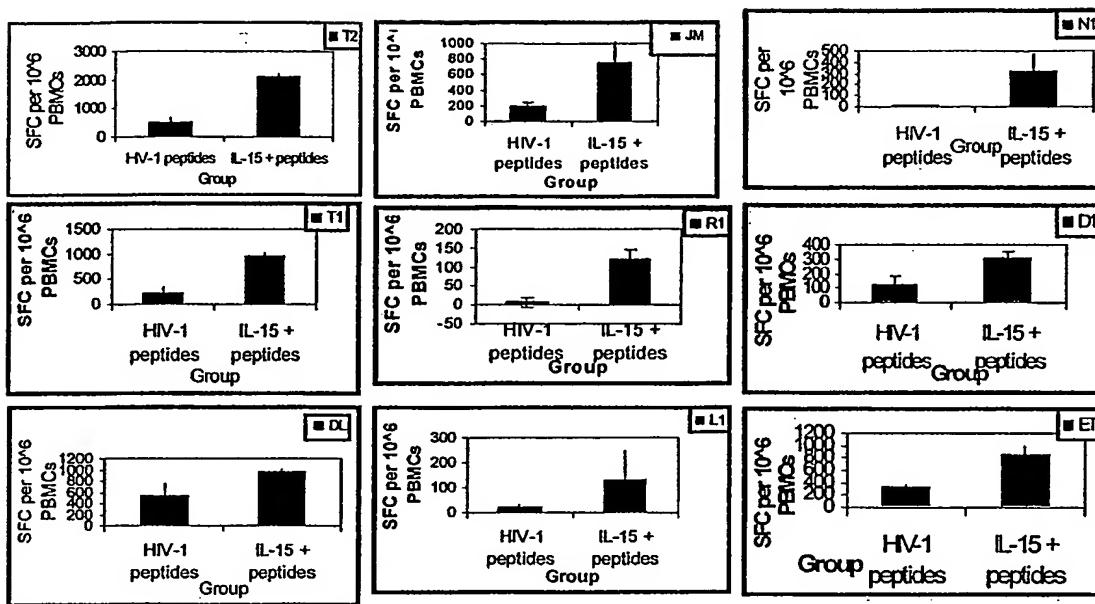


FIGURE 3B

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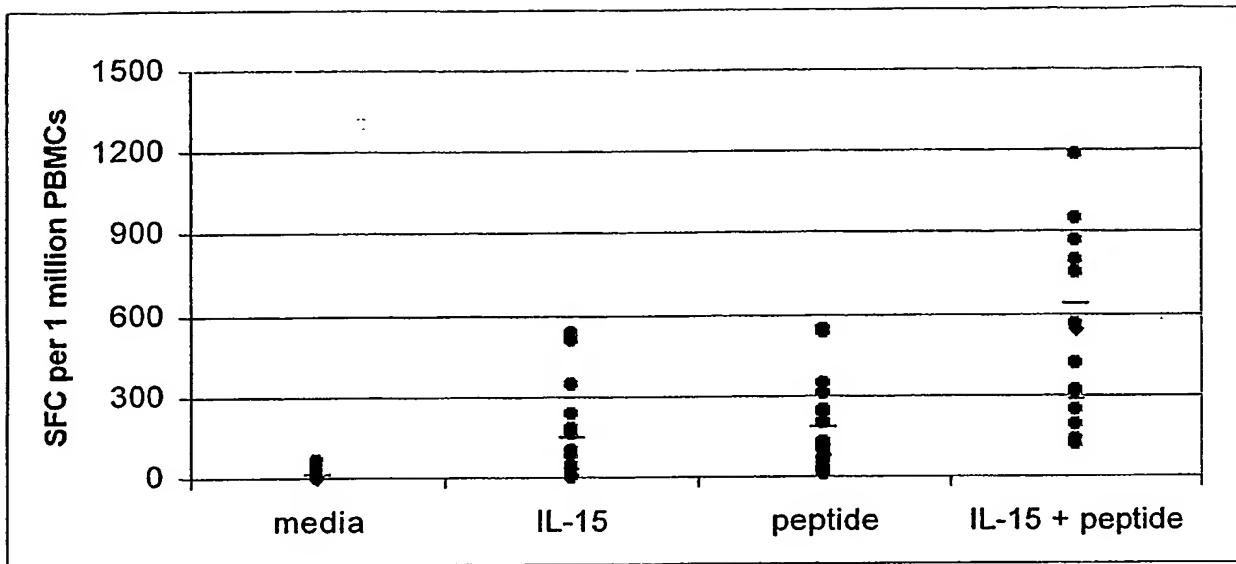


Figure 3C

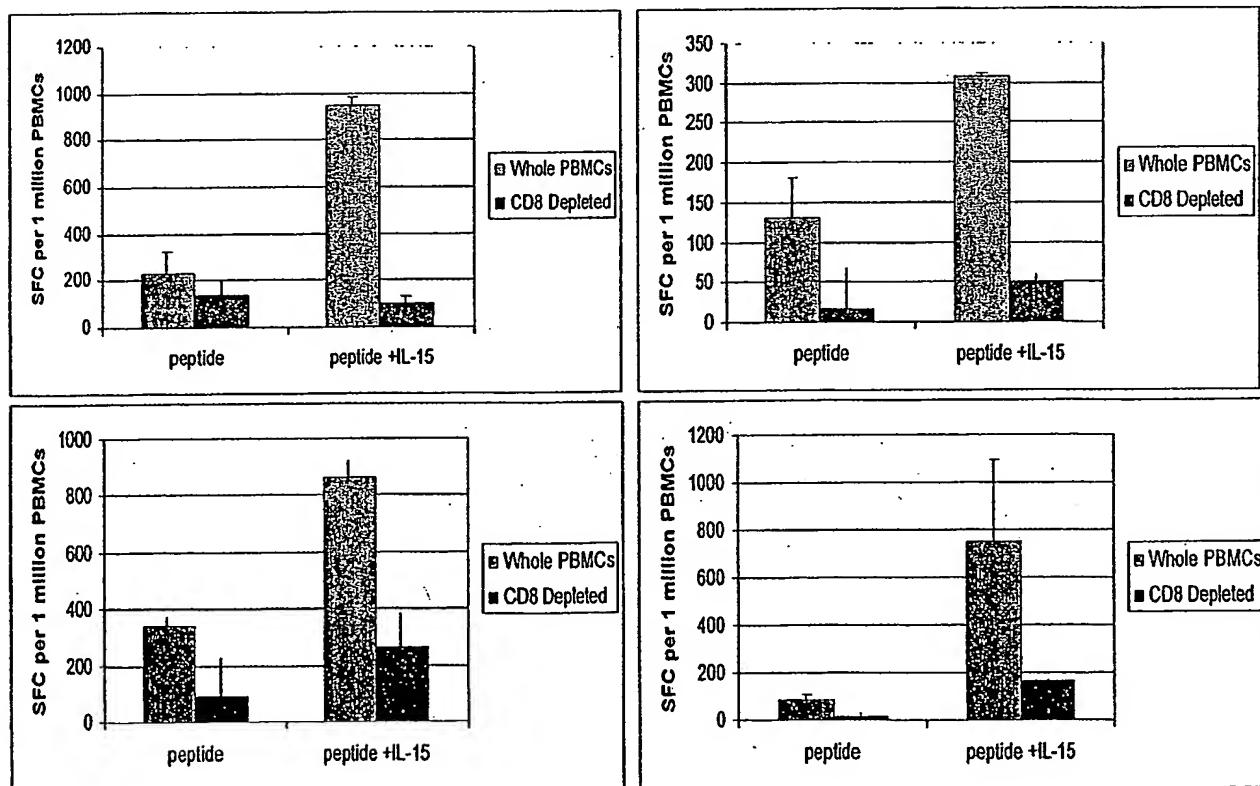
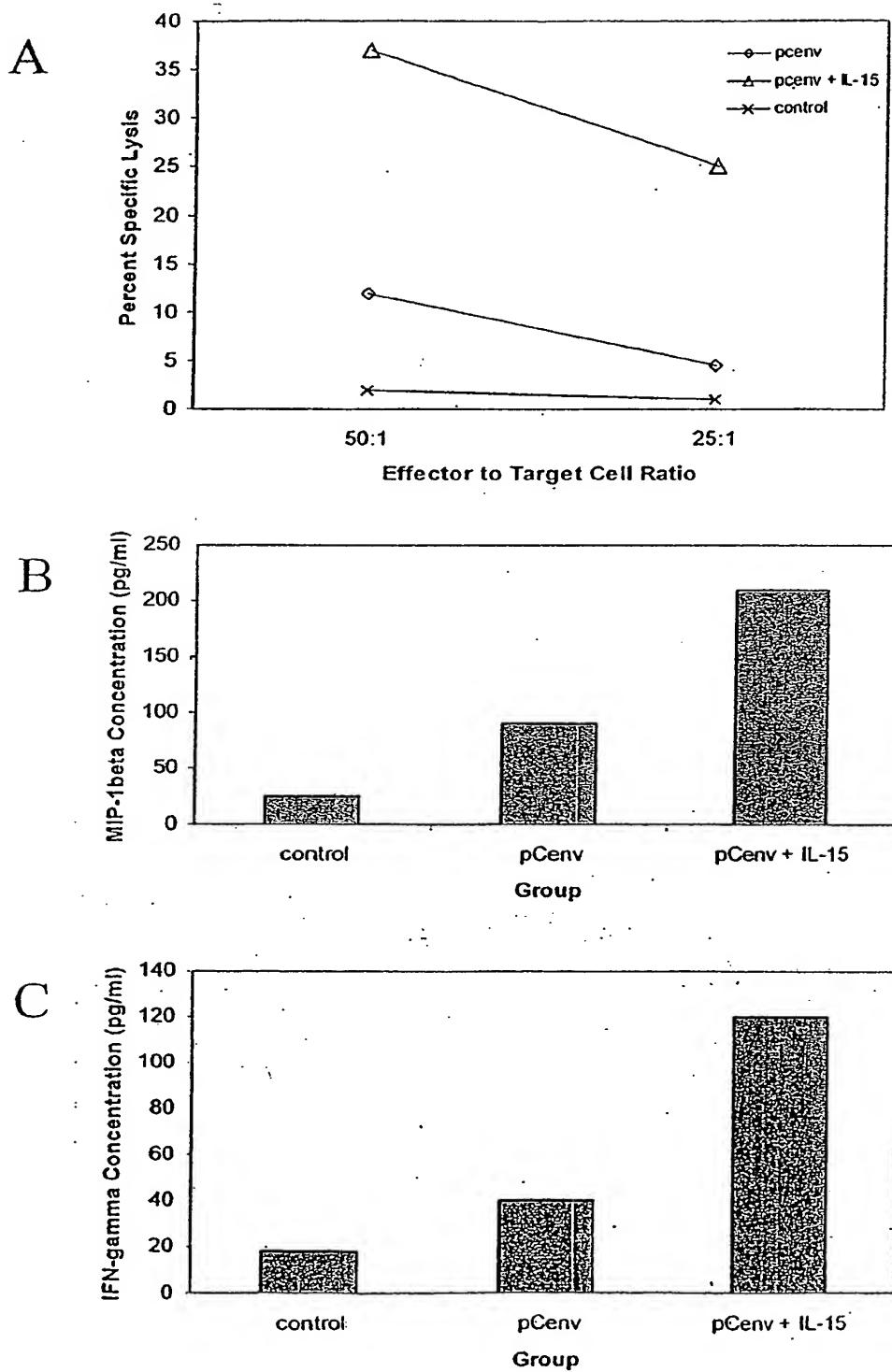


Figure 3D



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Figure 4

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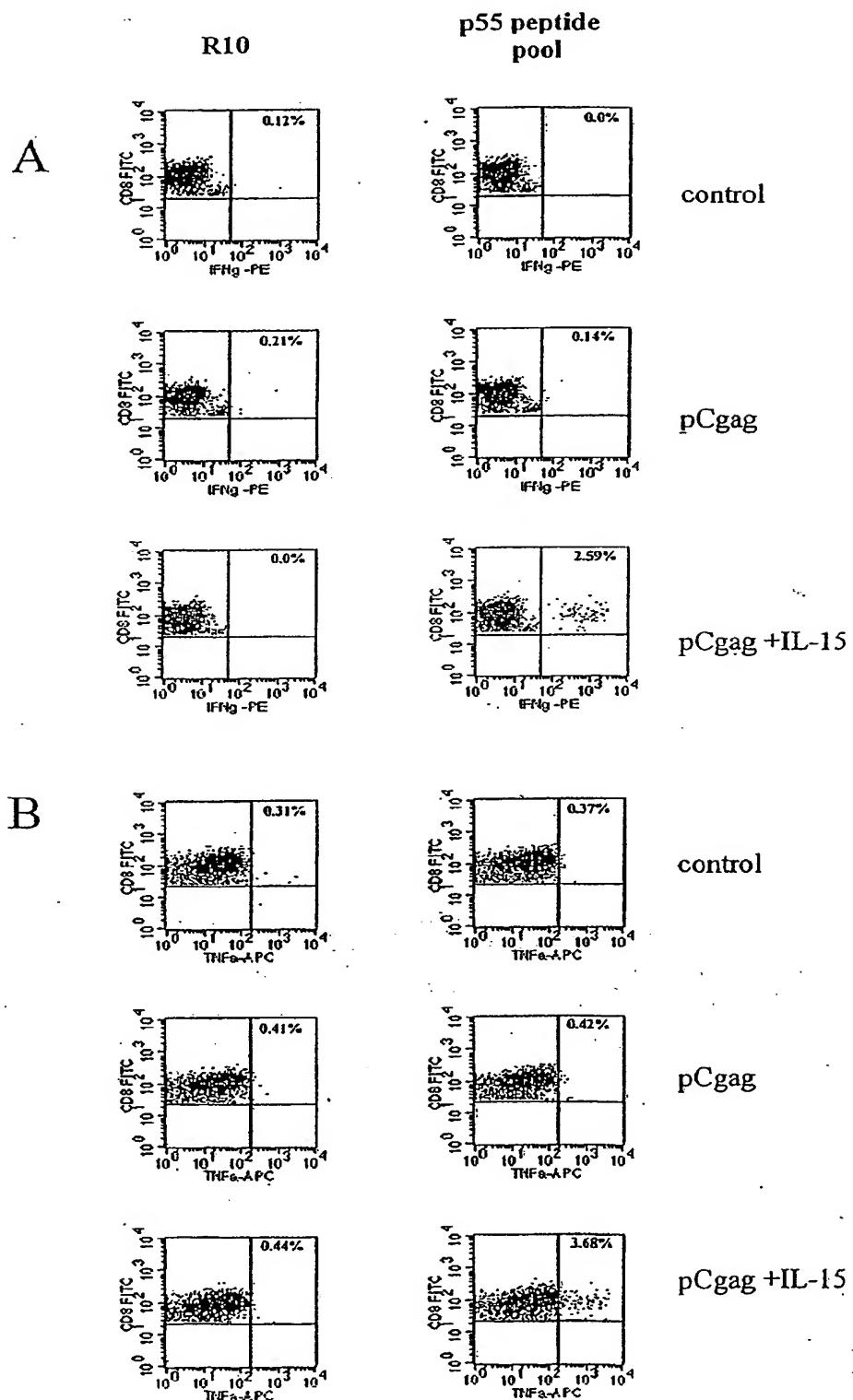


Figure 5

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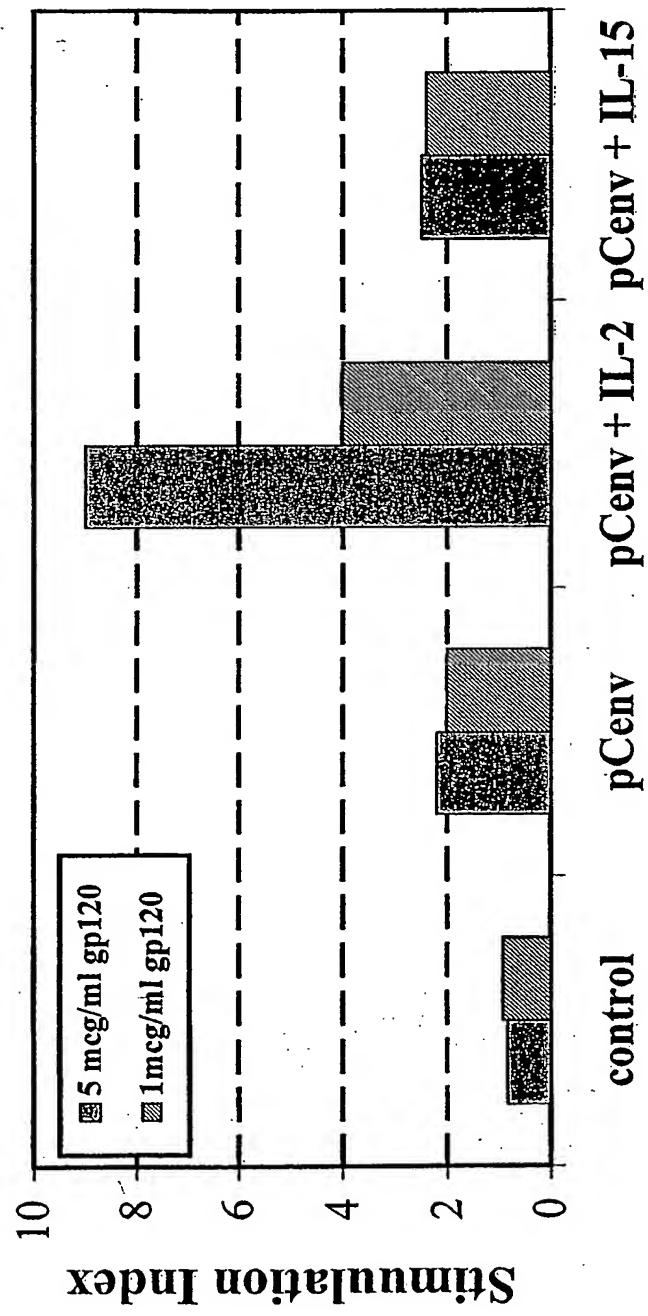


FIGURE 6

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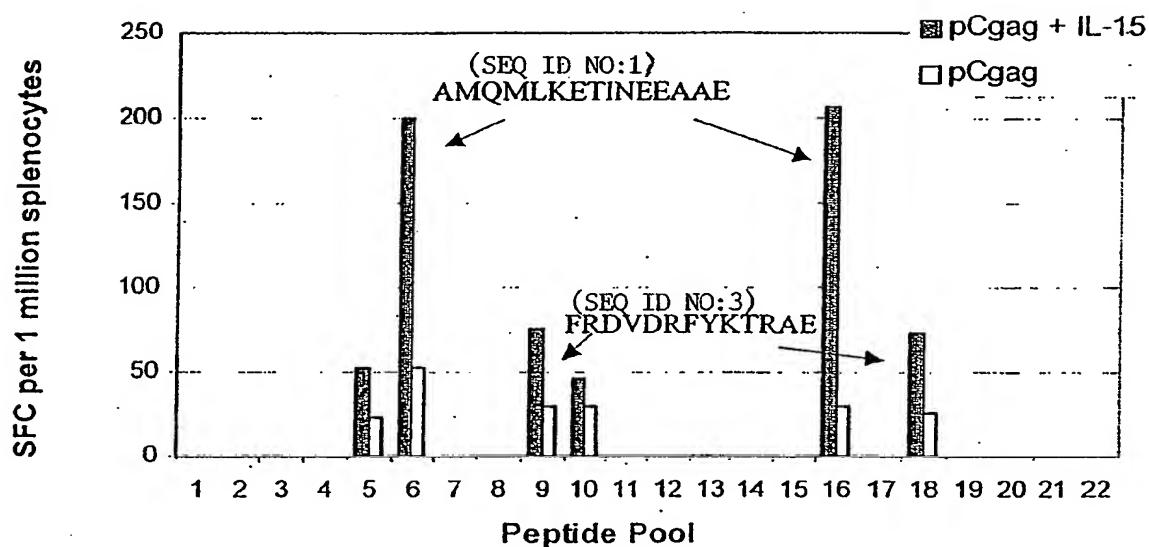


Figure 7

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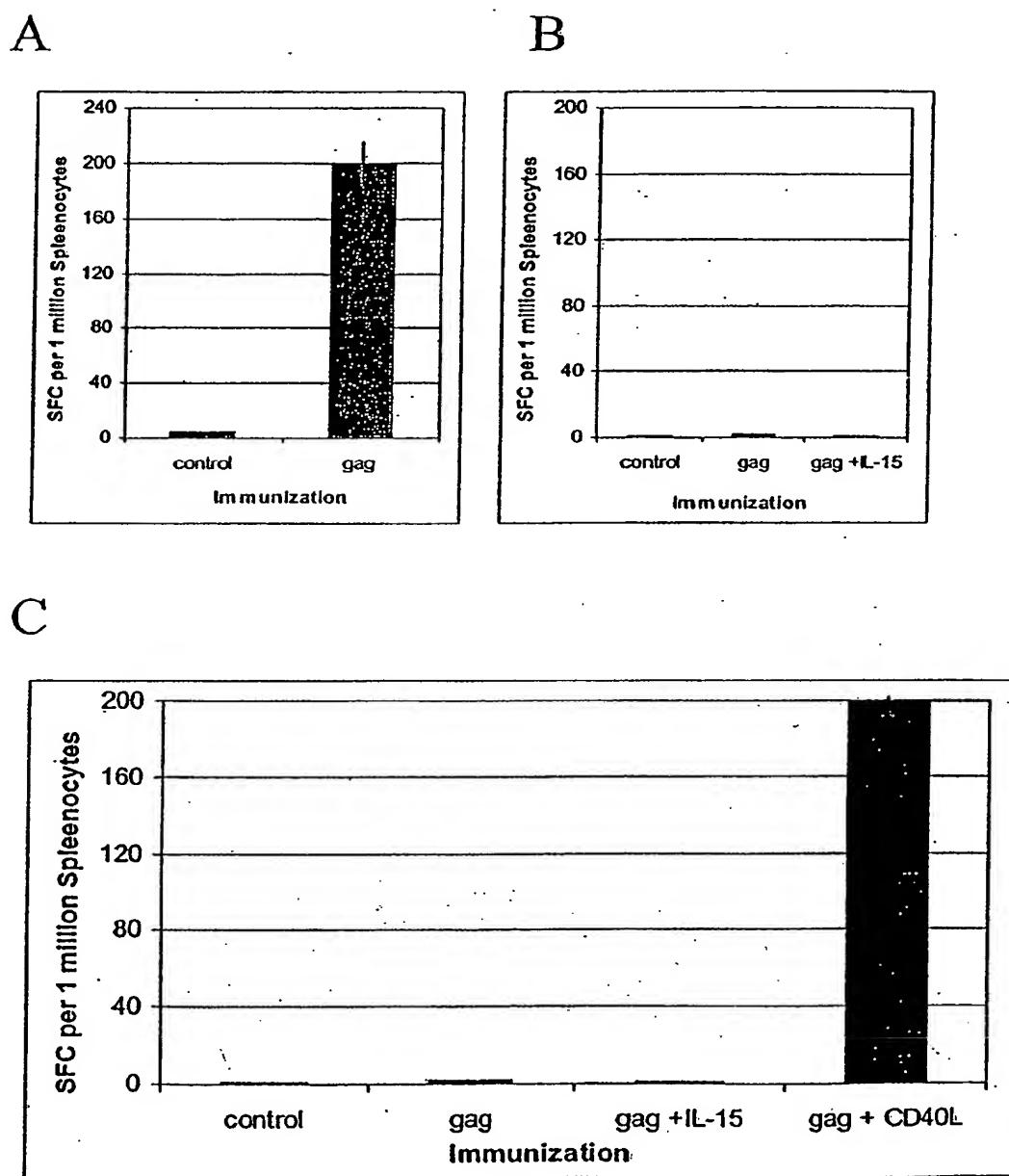


Figure 8

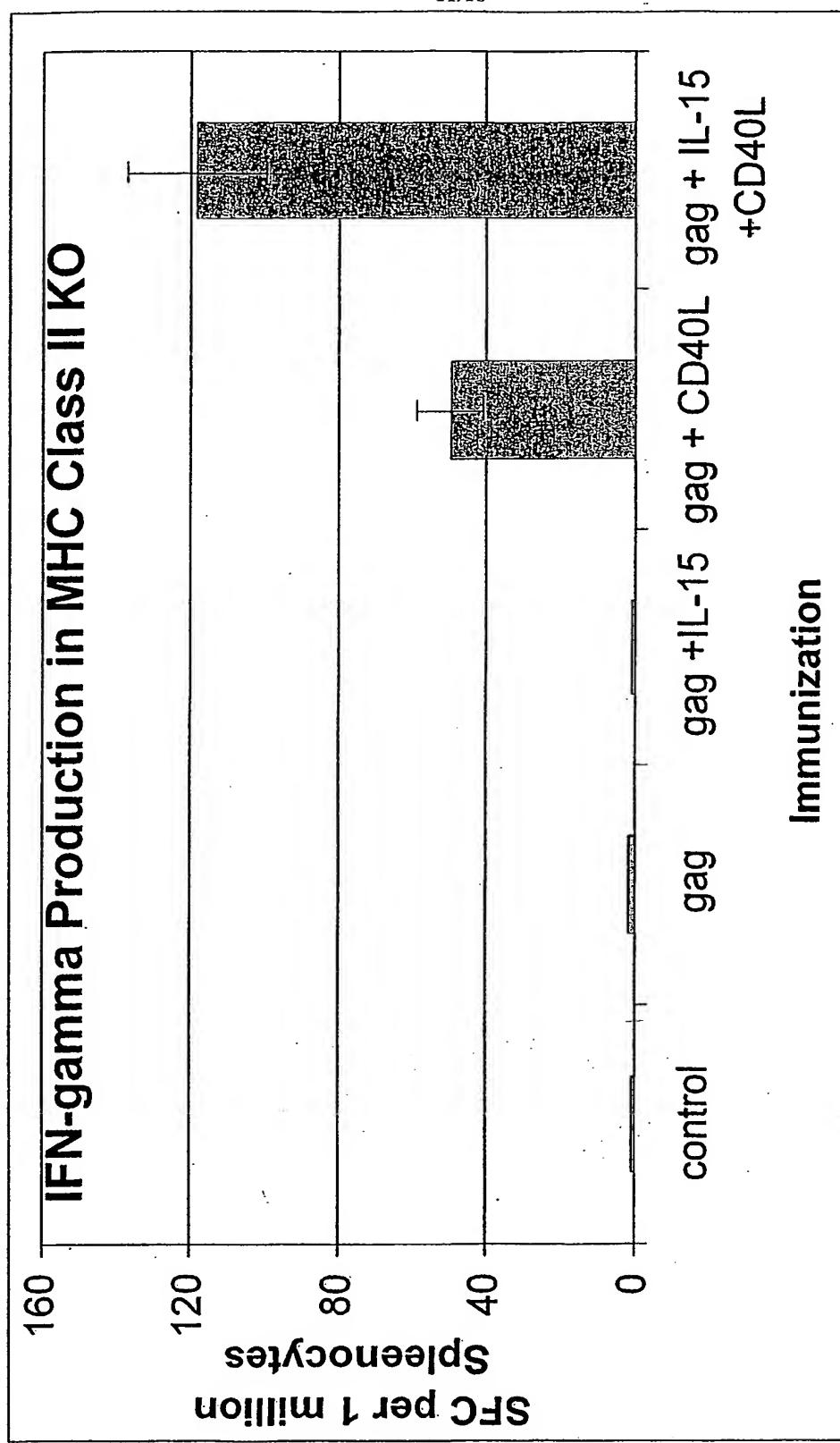


FIGURE 9

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# Strategy for Increasing Expression of IL-15 through Optimization of DNA Constructs for Immunization

- ❖ Primers are designed to amplify IL-15 from start of signal peptide, thus upstream inhibitory AUGs are not present in the final IL-15 message.
- ❖ Primers are designed to include a strong Kozak context (GCCGCCACCC).
- ❖ Removal of the C-terminus negative regulatory element using PCR antisense primer design

Primer Name	Sense/Antisense	Sequence 5' to 3'
Human IL-15 (LSP)	sense (SEQ ID NO:4)	GCCCCGGATCGAG GCCGCCACCCATGAGAATTTCGAAAACCACATTGAG
	antisense (SEQ ID NO:5)	ATCGGGGGTGGAGTCAAGAAAGTGTGATGAACATTGG
Macaque IL-15 (LSP)	sense (SEQ ID NO:4)	GCCCCGGATCGAG GCCGCCACCCATGAGAATTTCGAAAACCACATTGAG
	antisense (SEQ ID NO:5)	ATCGGGGGTGGAGTCAAGAAAGTGTGATGAACATTGG
Human IL-15 (SSP)	sense (SEQ ID NO:6)	GCCCCGGATCCGCCGCCACCATGGTATGGAAAGCTTGG
	antisense (SEQ ID NO:7)	ATCGGGGGATCC TCAAGAAAGTGTGATGAACAT

Legend: Restriction Site, Kozak, START, STOP CODON

FIGURE 10

## Strategy for Increasing Expression of IL-15 through Replacement of 48 amino acid Signal Peptide (LSP) with IgE leader

- ❖ Sense primers are designed to start after 48 aa LSP while antisense primer amplifies from stop site.
- ❖ Primers are designed to include a strong KOZAK context (GCCGCCACC).
- ❖ Sense primer is designed to contain the sequence for IgE leader sequence plus a ATG start site.

Primer Name	Sense/Antisense	Sequence 5' to 3'
Human IL-15-IgE	sense (SEQ ID NO: 8)	GGCCGCCACCATGGATTGGACCTGGATCTTATTTT
	(SEQ ID NO: 9)	AGTTGGCTGCTGCTACTAGCTTCAACTGGGTGAATGTAATAAGT
Macaque IL-15-IgE	antisense (SEQ ID NO: 5)	ATCGGGCTCGAG TCAAGGAGTGTGATGAAACATTGG
	(SEQ ID NO: 8)	GGCCGCCACCATGGATTGGACCTGGATCTTATTTT
	sense (SEQ ID NO: 9)	AGTTGGCTGCTGCTACTAGCTTCAACTGGGTGAATGTAATAAGT
	antisense (SEQ ID NO: 5)	ATCGGGCTCGAG TCAAGGAGTGTGATGAAACATTGG

Legend: Restriction Site, KOZAK, START, STOP CODON

FIGURE 11

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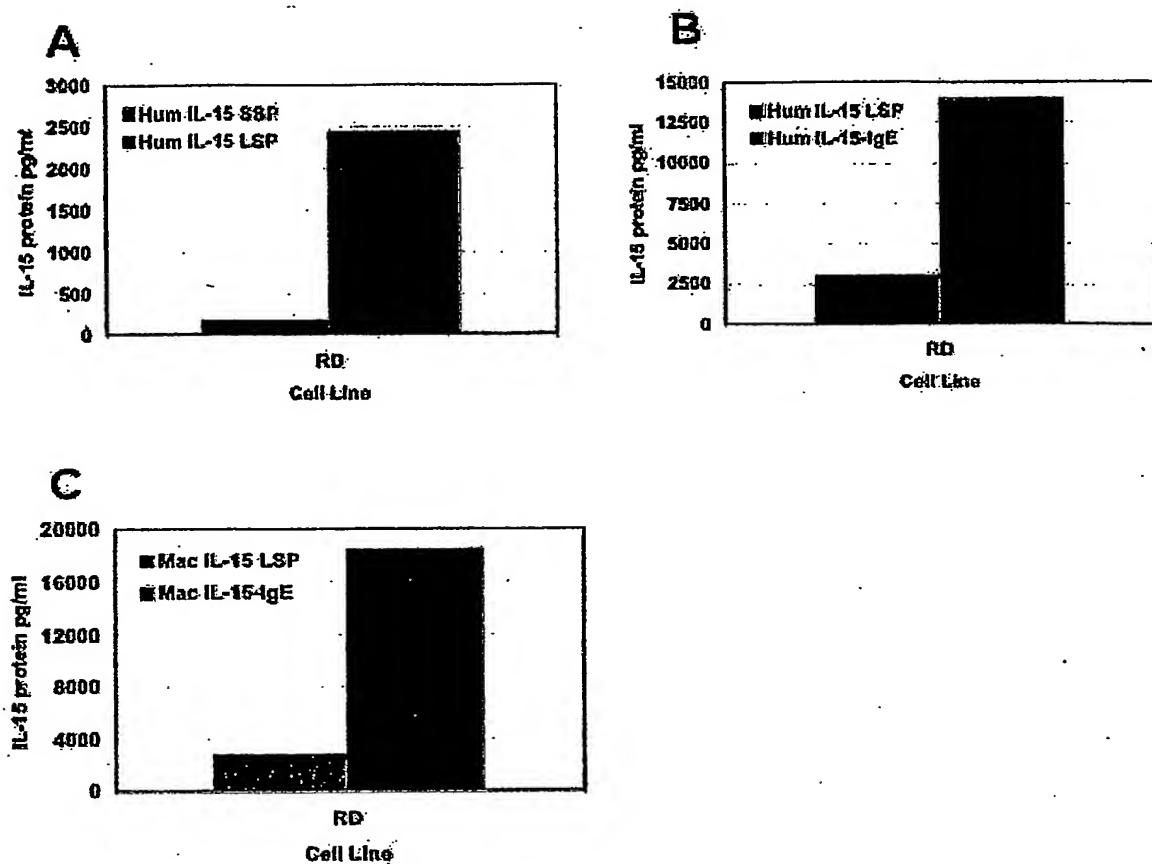


FIGURE 12

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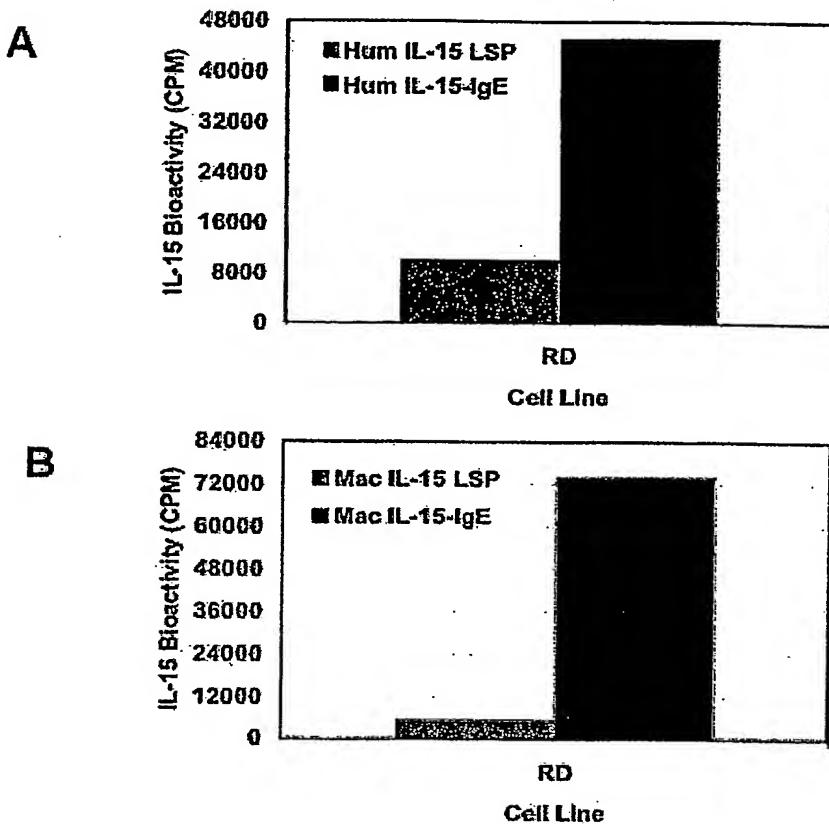


FIGURE 13

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## ***Immunization Schedule***

### Immunization Groups:

## Naïve

## Vector Control

## HIV-1 Gag

## HIV-1 Gag/IL15 constructs

*Combinations of 100 µg IL15 Constructs, 50 µg GAG,  
Each injection, intramuscular*

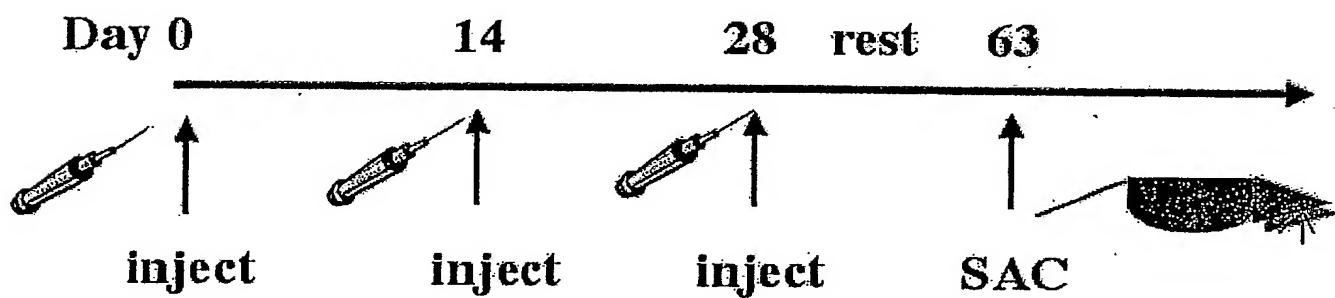


FIGURE 14

**SUBSTITUTE SHEET (RULE 26)**

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Restimulation of antigen-specific IFN- $\gamma$  production 5 Weeks Following  
the 3rd immunization of HIV-1 Gag in Balb/C mice  
**Effect of IL-15 Constructs**

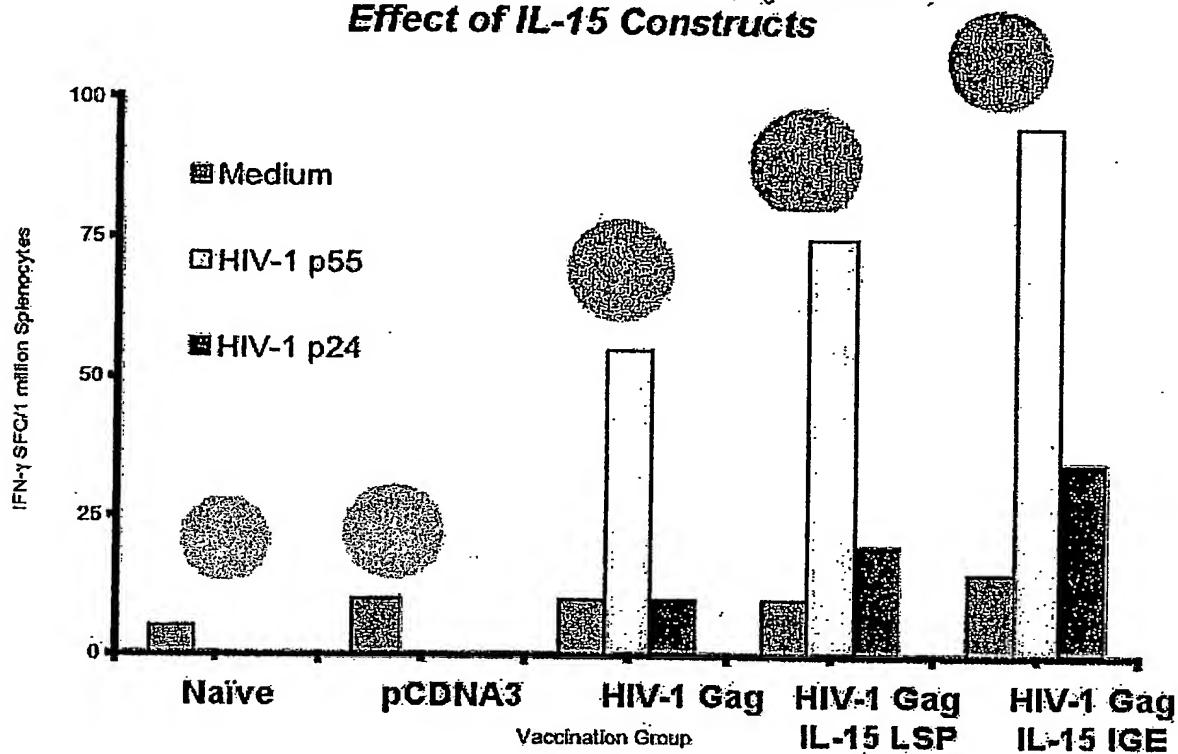


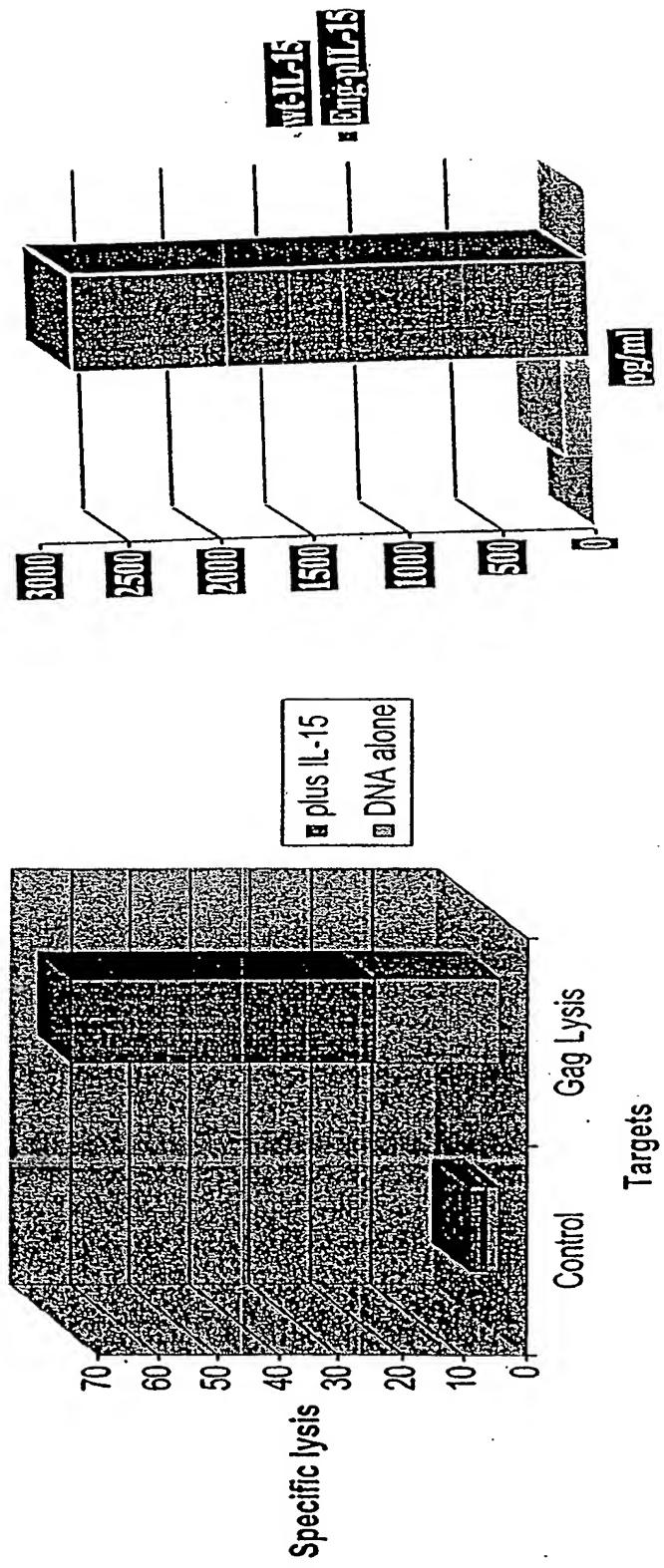
FIGURE 15

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An Engineered IL-15 Plasmid Vaccine  
(Kozak, AUG's removed, UTR's removed & other-30-  
50X better expression)

Enhances CTL response in Vivo

Mice were immunized with HIV-1 gag expressing DNA



Grabstein et al. (1994) Science 264:965-968, Bamford et al., 1996 PNAS 93:2897-2902  
Bamford et al., (1998) J. Immunol 160:4418-4426, Kozak et al., (1991) J. Cell Biol. 115:887-903

FIGURE 16

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